FINAL REPORT

INSTRUMENTATION FOR QUANTITATIVE EVALUATION OF QUALITY COMPONENTS TO SUPPORT THE HERB, FRUIT AND VEGETABLE HORTICULTURE SECTORS

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Instrumentation for quantitative evaluation of quality components to support the Herb, Fruit and Vegetable Horticulture Sectors
AFIF Project # 97000207

a) Summary

The instruments purchased for the evaluation of quality components in horticulture fruit, vegetable and herb crops are: texture analyzer, mass sugar analyzer, HPLC, HPTLC. Since the Crop Science Dept. had a colorimeter which had been underutilized, that piece of equipment was not purchased. Our departments (Crop Science and Horticulture Science) have since been amalgamated. That colorimeter is in the process of being translocated to the horticulture labs. Both Dr. Waterer's and Bob Bor's programs heavily use this piece of equipment. The high Cdn/US $ exchange combined with unexpected higher costs of the equipment resulted in the majority of the funds utilized for these 4 instruments. The equipment was purchased from the summer, 1998 to as late as the summer, 1999. Inspite of considerable time and effort to set up and run the equipment, results are already available (see below). Many grants have benefited from the presence of these instruments. However, two areas still require development as outlined in the original grant: 1) servicing a greater number of clients, and 2) creation of a coordinated "center" for quality evaluation. Departments such as Applied Microbiology and Food Science as well as Agriculture Engineering, Environmental Test Labs and Grain Quality/chemistry should be more actively involved. It is hoped that by the next report, progress on these 2 areas will be addressed.

b) Technical Report

i) Background and Objectives

The ability to accurately, reproducibly and objectively evaluate quality components such as colour, medicinal compounds and sugar content of fresh, stored and processed horticultural commodities is critical to the vegetable, fruit and herb industry's efforts to continued growth and expansion. Equipment purchased to fulfill this goal will be housed at the Dept. of Plant Sciences, University of Saskatchewan. It will build on existing facilities and equipment, coordinate with the Dept. of Applied Microbiology and Food Science, and result in a strengthening of research and development programs being conducted by the University of Saskatchewan in support of Saskatchewan's horticulture industries.

For a detailed description of the equipment and uses, please see the original grant application.

Objectives:

- To purchase the following equipment: HPLC, HPTLC, Mass Sugar Analyzer, Texturemeter.
- To utilize that equipment to address industry needs.
- To develop a plant quality analysis center with a listing of equipment, use and location.

ii) Project Activities and Achievements

All of the equipment, attachments and supplies have now been purchased:

- Mass Sugar Analyzer: YSI Model 2700S Biochemical Analyzer, Single Channel
- Texture meter: Model TA.XT21, 25 kg capacity, Texture Technologies Corporation.
- HPTLC: Camag Scientific Inc., cat. No. 022.9543C
- HPLC: Waters 600E Multisolvent delivery system

However, not all of the equipment has been utilized since the HPTLC was just received last summer. The Mass Sugar Analyzer has also not been utilized but harvested fruit from 1999 will be analyzed. Note: since the dept. already had an identical colorimeter which was underutilized, it was decided not to purchase this piece of equipment. In addition, the US/Cdn exchange rate was the highest on record when most of the equipment was purchased and the equipment such as the texturemeter cost much more than expected. Had the colorimeter also been purchased, we would have gone over budget.

iii) Identification of facilities and equipment problems and performance summary

Equipment is housed at the University of Saskatchewan, Dept. of Plant Sciences and supporting facilities within the department include: fume hoods; flow hoods; drying ovens; ultra-low freezers; freeze-dryer; rotovaporator; extraction facilities; growth chambers/greenhouse; bench space with water, vacuum and air; various centrifuges including ultra and analytical; Gas Chromatograph; balances; ph meters; chemical and material supplies; etc. etc.

No equipment problems have been detected at this point and the equipment has performed satisfactorily.

iv) Number of clients served

Requests from the vegetable and fruit industries have not yet been received. In addition, the HPTLC and mass sugar analyzer have not yet been utilized. In terms of the Herb & Spice Industry: this HPLC has taken a significant load off of the other 2 HPLC's in the department which are used for both research and industry purposes. Therefore, more clients can be served in total (about 15-20 clients, see section v. below).

v) Impact on the industry

The following ADF projects (supporting and servicing the industry) have utilized the equipment outlined to fulfill those activities:

- ADF # **Domestic Fruit Development Program.**
- ADF # 95000307 **Pyrethrum: An Outlook for Saskatchewan**

The Herb Research Program has utilized the equipment for the following projects:

- ADF No. 96000097 **Immunostimulants from Saskatchewan Indigenous Plants** (this project is of confidental nature)
- ADF No. 96000233 **High quality standards for raw and processed herbs,** a prerequisite for strong herb industry in Saskatchewan. This project includes provision of analytical services on a cost recovery basis to medicinal herb industry. Over the last year over 150 samples were analyzed by the Saskatchewan Herb Research program; about 10-15% of the samples were analyzed using equipment purchased by the AFIF instrumentation grant.
- AFIF Project **Specialized Crops Spoke - Quality Management** (Univ. account No 7-72767; don't have AFIF account no.).

The HPLC and HPTLC will be used in the near future for the ongoing projects in the Saskatchewan Herb Research Program:

- ADF No. 98000292 **Development of methods to identify authentic and superior seed of commercially important Echinacea species: an interprovincial study.**
- ADF No. 97000334 **Use of traditional Chinese herb medicines in study of Saskatchewan wild plants for natural anticancer substances.**

Native Fruit Program has utilized this equipment in the following projects:

- ADF Project 96000249 - **Fruit Quality Screening for the Development and Evaluation of Saskatoon, Chokecherry and other Native Fruit Cultivars and Germplasm.**
- ADF project #98000291- **Effects of ozone treatments on shelf-life extension, physiology and quality of fresh saskatoon berries.**
- AFIF Project #97000171 - **PostHarvest Specialist Program.**
- AFIF project # 98000145 - **Determination of the Effects of Hot and Cold Water Washing and Drying on Shelf Life of Fresh Saskatoon Berries.**

vi) Impact on research and breeding programs

- **Texture Analyzer** (TA.XT2, Texture Technologies Crop., Scarsdale, NY)

  Location: 2C83, Agriculture Building
  Users: Native Fruit Development Program, Postharvest Specialist Program, Class Demonstrations in Horticulture, Domestic Fruit Development Program

  Saskatoon berries:
  Postharvest program: To assess textural changes in immature and mature saskatoon berries stored under modified atmosphere conditions.
  Wash/dry project: To examine the effect of hot and cold water washes followed by drying on the textural quality of fresh-pack saskatoon berries.

Class Demonstrations on Apples:
Provided by Wendy Pomedli for Tom Ward

Potatoes:
Postharvest program: to investigate changes in textural quality of red Norland potatoes during storage:

Texture analysis

Average-sized potatoes were selected from storage and washed with any surface moisture removed using paper towels. Potatoes were placed on a cutting board along their longitudinal axes. Sections 2 cm thick along the middle region were marked from the stem to the bud end and cut using a sharp knife. Texture was evaluated by puncturing the surface of the section with a 2 mm diameter probe attached to a **TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY)**. Five punctures were made on the surface of each potato (Figure 1), one each at the stem end, pith and bud end and two in the parenchyma tissue on each side of the tuber outside of the vascular ring. The rationale for puncturing at various points was to minimize variability within the tuber. The probe was allowed to puncture halfway into the tissue (i.e. 1 cm from the surface). Peak force was used as an index of firmness. Peak force is the maximum force required to puncture the tissue. High peak force is indicative of a firm potato and low peak force suggests a less firm potato. All experiments were done in duplicate. Three potatoes were analyzed in each replicate.

Table 1. Effect of storage period on peak force of regular and dark red Norland potatoes

Storage month	Peak force (N)	
	Dark reds	Regular reds
December	5.86*ab*[2]	6.37*a*[1]
February	6.07*a*[1]	5.23*b*[2]
April	5.50*b*[1]	5.58*b*[1]

Means in columns for a given strain followed by the same letter are not significantly different from each other (p• 0.05). Means in rows for a given property followed by the same number are not significantly different from each other (p• 0.05).

NB: Information extracted from Interim Report

- **Mass Sugar Analyzer (YSI Incorporated, Yellow Springs, OH)**
  Location: 2C83
  Users: Fruit Development Programs to use in the fall/winter 1999/2000.

- **HPLC (High Performance Liquid Chromatograph)**

In December 1998, the single buslace kit was converted to a multi buslace kit allowing the departmental gas chromatography (GC) to be controlled from the same software as the HPLC ($1660). This has streamlined operations and maximized the benefits obtainable from the software purchased for the HPLC.

Specifically, the Native Fruit Development Program has utilized the HPLC to develop methodology for anthocyanin analysis. Anthocyanins are essentially colour compounds present in fruit. This method will be used to assess anthocyanin contents of fruit of various Saskatoon genotypes and possibly to evaluate other fruit such as chokecherry and black currant. In the future, methodology for examining organic acid contents will also be developed. Again, fruit of various Saskatoon genotypes and possibly chokecherry and black currant will be assessed. These analyses will occur over considerable time allowing crops from several years to evaluated. The availability of the departmental HPLC has made this research possible.

Preliminary method development included assessing HPLC conditions in detection of an anthocyanin standard and anthocyanins in saskatoon sample extracts, identifying the peaks in a mixture of standards, and assessing differences among various amounts of initial material extracted. Preliminary results are presented in the following tables and figures. Method development is continuing to determine appropriate amounts of material extraction, dilution factors, and injection volumes.

Retention times of anthocyanin standards:

Injection Volume (uL)	Cyn-3-Gal	Cyn-3-Glu	Pel-3-Glu	Peo-3-Glu
20 uL (individually)	5.863	6.906	8.294	9.335
20 uL (mixture)	5.923[1]	6.966[1]	8.345	9.378
15 uL (mixture)	5.940[1]	6.981[1]	8.346	9.366
10 uL (mixture)	5.920	6.955	8.320	9.345

[1] These peaks were overlapping.

Retention times of various material extraction amounts.

Sample	Cyn-3-Gal	Cyn-3-Glu	Pel-3-Glu	Peo-3-Glu
25 g	5.950	7.002	7.659	-
20 g	5.987	7.033	7.688	-
15 g	6.632	7.593	8.220	-
10 g	6.603	7.563	8.183	-
Stds 20uL (Mix.)	5.863	6.906	8.294	9.335

Herb Research Laboratory: Use of HPLC instrument (Rm. 2C79)

Water's HPLC instrument has been installed in the fall of 1998 and has been used mainly by the Herb Research Program. Current uses include development of analytical methodology for phytochemical quality evaluation of two medicinal plants, *Polygala senega* (senega root) and *Calendula officinalis* (marigold). The objective of this work is to determine optimal conditions for separation and quantitation of saponin marker compounds in senega root and various flavonoid compounds in flowerheads of calendula.

This instrument is also used for semi-preparative work, isolation and partial characterization of active compounds from plants, such as senegin II and IV from *P.senega*. In the near future instrument will also be used for analysis of sea buckthorn leaves, berries and seed for various flavonoid compounds. This is a joint project between the PFRA Shelterbelt Center in Indian Head and the Department of Plant Sciences.

The following is an excerpt from our ADF project, Pyrethrum: An Outlook for Saskatchewan:

3.5 Analysis of pyrethrins in pyrethrum (*Tanacetum coccinium*) flowers

Experimental

Materials

Standard pyrethrum extract (~ 25 % pyrethrin I + pyrethrin II) was obtained from Fluka Chemical Corp., Sigma-Aldrich Canada Ltd., Oakville, ON, Canada. Petroleum ether (b.p. 38.5 - 56.5 °C), used for the extraction of pyrethrins, was of A.C.S. grade and purchased from Fisher Scientific Ltd., Nepean, ON, Canada. OmniSolv tetrahydrofuran (EM Science, Gibbstown, NJ, USA) and HPLC grade n-hexane (Fisher Scientific Ltd., Nepean, ON, Canada) were used in liquid chromatography analysis.

Sources of pyrethrum flowers

The flowering parts of seven pyrethrum samples, grown locally in Saskatchewan and harvested on June 24 1997, were used for extraction of pyrethrins. In addition, two pyrethrum samples grown in Kenya were analyzed for comparison purposes.

Extraction of pyrethrins

1.5 to 1.7 grams of dry finely ground pyrethrum flowers were extracted with 75 mL petroleum ether in a water shaker bath (20 °C) for 3.5 hrs. The flasks were sealed, to prevent evaporation of solvent, and covered with aluminum foil in order to prevent degradation of pyrethrins caused by ambient light. The resulted dark yellow-greenish solutions, containing pyrethrins, were filtered through a Whatman filter paper (grade 202, Whatman Inc., Clifton, NJ, USA) and stored in a dark place at 4 °C until they were analyzed.

High Performance Liquid Chromatography (HPLC) analysis

HPLC analyses were performed on a Waters liquid chromatograph (Waters Corporation, Milford, MA, USA) equipped with a Waters 996 photodiode array detector (PDA), a Waters 600E multisolvent delivery system and a Waters 717 plus autosampler. Data were collected using Millennium^{32} chromatography manager, version 3.05 (Waters Corporation, Milford, MA, USA). Separations were performed on a Waters Nova-Pak® Silica 60 Å 4 µm (150 mm x 3.9 mm i.d.) column with a Sentry Guard Nova-Pak® Silica 60 Å 4 µm (20 mm x 3.9 mm i.d.) pre-column. Chromatographic runs were carried out using gradient conditions: start to 21 min, 96 % n-hexane - 4 % tetrahydrofuran, flow rate 0.7 mL / min; 21 to 21.50 min, 100 % n-hexane, flow rate 1 mL / min and remained there until 24.50 min; 24.50 to 25 min, system returned to initial conditions. Standards and samples tested were dissolved in petroleum ether and filtered through a 4 mm Nylon 0.45 µm filter (Waters Corporation, Milford, MA, USA) prior to being injected onto column. The injection volume was 10 µL and the column effluent was scanned from 200 to 350 nm and monitored at 229 nm. A series of six standards were used for establishment of calibration curve containing 0.1, 0.2, 0.4, 0.8, 1.0 and 1.6 mg / mL total pyrethrins.

4.0 Results

a) Pyrethrin Analysis

Pyrethrins in pyrethrum flowers exist as pyrethrins I (Jasmolin I, Cinerin I and Pyrethrin I) and pyrethrins II (Jasmolin II, Cinerin II and Pyrethrin II). Identification of individual pyrethrins was achieved by combining photodiode array detection data and data available in literature (McEldowney and Menary, 1988). Retention time and spectroscopic data for individual pyrethrins are presented in Table 1. A typical chromatogram of standard pyrethrum extract is shown in Figure 1.

Table 1: Chromatographic and spectroscopic data for individual pyrethrins

Compound name	Order of elution	Retention time (min)	Maximum absorption wavelength (nm)[1]	Maximum absorption wavelength (nm)[2]
Jasmolin I	1	4.2	220.4	219
Cinerin I	2	4.5	220.4	220
Pyrethrin I	3	5.2	222.7	222.5 – 223
Jasmolin II	4	9.8	228.6	229
Cinerin II	5	10.8	229.8	229
Pyrethrin II	6	13.4	227.4	227 - 228

[1]As determined by this study
[2]As reported in McEldowney and Menary, 1988

Presence of all six pyrethrins was confirmed in all samples studied with the exception of Robinson 18#21, Robinson 1#4 and Robinson 32#35 samples in which cinerin I was absent. An unidentified peak at 5.8 min was present in all Saskatchewan growing plants but absent from those grown in Kenya. Pyrethrin I was the dominant of the six pyrethrins for Robinson 18#21, Robinson 7#10, Robinson 25#28 and Kenyan #B samples and pyrethrin II was the dominant compound in the remaining samples. Typical chromatograms of a locally grown and one grown in Kenya pyrethrum plants are shown in Figures 2 and 3.

Total peak area of pyrethrins (pyrethrins I and II) was used for the determination of the amount of pyrethrins present in the nine samples studied due to lack of individual standards. In addition, determination of the percentage of pyrethrins I and II in the samples was not possible since the exact composition of pyrethrins I and II in the standard was not known (standard contained ~ 25 % pyrethrins I and II).

A linear relationship was obtained between total pyrethrins area and concentrations of the six standards used ($R^2 = 0.998$; Figure 4). Amounts of total pyrethrins expressed in % weight by weight in samples studied are shown in Table 2.

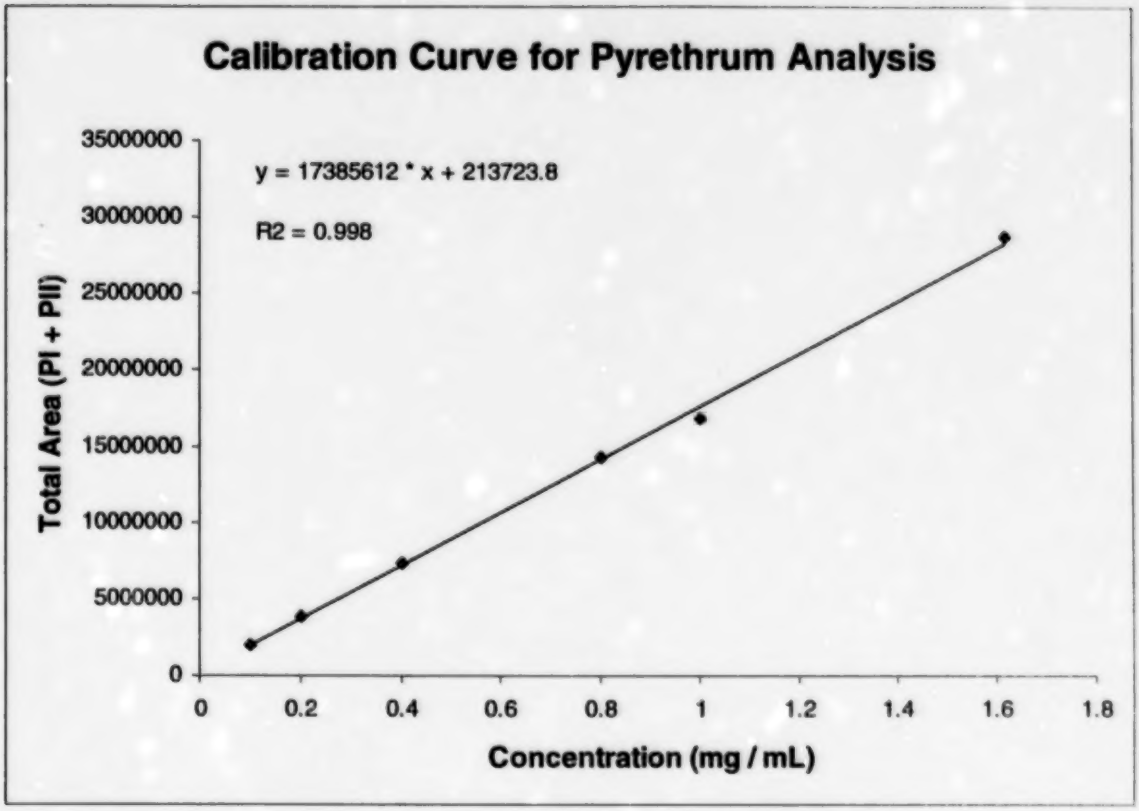
Calibration Curve for Pyrethrum Analysis
y = 17385612 * x + 213723.8
R2 = 0.998
Total Area (PI + PII)
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Concentration (mg / mL)

Table 2: Amount of total pyrethrins (PI + PII) present in flowers of seven locally grown and two grown in Kenya pyrethrum plants.

Sample ID	Dry weight (g)	Weight used for extraction (g)	Volume of extraction solvent (mL)	Concentration (mg / mL)[1]	% w / w
Robinson 1#4	15.33	1.4963	75	0.829	42.56
Robinson 5#8	12.66	1.4883	75	0.555	23.77
Robinson 7#10	12.24	1.4906	75	0.381	15.72
Robinson 18#21	17.50	1.6785	75	0.672	31.30
Robinson 19#22	8.83	1.5347	75	0.874	24.56
Robinson 25#28 (light)	3.46	1.5268	75	0.409	4.56
Robinson 32#35	4.90	1.4697	75	0.365	6.22
Kenyan #A	0.443	0.2633	20	0.631	11.90
Kenyan #B	0.283	0.2168	20	0.386	3.15

[1]As determined from calibration curve

Sample calculation for Robinson 1#4:
Total area: 14622429
From equation of line y = 17385612 * x + 213723.8
x = 0.829 mg / mL
0.829 mg / mL * 75 mL * 1 / 1.4963 g = 41.54 mg / g

thus total pyrethrins in dry total weight: 41.54 mg / g * 15.33 g / 1.4963 g = 425.60 mg /g

therefore % w / w = 425.60 mg / g * 1 g / 1000 mg * 100 = 42.56 % (w / w)

Reference: McEldowney, A. M. and Menary, R. C. 1988. Analysis of pyrethrins in pyrethrum extracts by high-performance liquid chromatography, *Journal of Chromatography*, **447**, 239 – 243.

- **HPTLC (High Performance Thin Layer Chromatograph)**

The HPTLC system is used regularly in analysis of herbs and herbal products for qualitative, quantitative and preparative work. Qualitative analyses include fingerprint tests, which are used for rapid identity checks. It determines whether material (dried herb or an extract) is derived from a defined botanical species. An important advantage of the HPTLC system in comparison to conventional TLC system is parallel evaluation and comparison of multiple samples with high accuracy.

Quantitative analyses include content determination of important marker compounds, while preparative analysis include isolation of single pure compounds from complex mixtures of natural compounds contained in each herb.

The limited HPTLC system, purchased from Camag Scientific Inc., North Carolina (Swiss made) in April 1999 is suited for semi-automatic sample application bandwise to the plate (Linomat IV) and recording and documentation of planar chromatograms (Video Store 2). By applying samples in narrow bands, this instrument ensures the highest resolution attainable with the TLC system selected. Instrument allows relatively large sample volumes to be applied uniformly in narrow bands of selectable lengths. Linomat IV is interfaced with a video camera for obtaining pictures as the plate is developed and after its development. Linomat IV sample applicator has recently been installed and is currently being tested using herbal extracts of Saint John's Wort as a model system.

Video Store 2 has not been installed yet since funds were insufficient to buy the complete Image Documentation System Video Store 2, capable of acquiring, printing and archiving images of thin-layer chromatograms. A high resolution, highly sensitive CCD video camera, a computer with frame grabber and monitor and powerful Video Store software were purchased with AFIF funds. However, a lighting module with UV lamp (254 nm "short wave" and 366 nm "long wave") integrated with camera bellows and video camera support, Reprostar 3, could not be purchased. The idea was to either secure funds from other source for buying original Camag's Reprostar 3 unit (about US$ 6,000), or to have a piece custom made locally. It is anticipated that the solution will be found within 6 months.

Once installed, The HPTLC system will have a broad application in herb research. For example for:
1) fast assessment of the influence of various agronomic practices on composition of medicinal ingredients;
2) optimization of harvesting time and post-harvest handling methods for each of commercially viable medicinal plants;
3) optimization of processing parameters for a certain herb in order to obtain highest quality extract;
4) quick comparative analysis of large number of commercially available herbal products.

c) Personnel

No personnel have been hired on this grant. The university's in-kind contribution represents the human resource portion of the project. The university's personnel include: Dr. Branka Barl, Dr. Shika Agblor, Wendy Ziehl, George Katselis, Rick Sawatzky, Forrest Scharf, Bob Bors, and Doug Waterer.

d) Equipment

See above.

e) Project Photos

No photos were taken at this time.

f) Innovation Fund Acknowledgement

It is too early for this but AFIF will be acknowledged in research & extension reports.

g) Project Public Information Materials

Not applicable